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THE DIRECT DETERMINATION OF SUCROSE IN THE PRESENCE OF REDUCING SUGARS

The methods proposed for the direct determination of sucrose in the presence of reducing sugars have not proved quite successful and none has come into general use. They are based on the destruction of the rotary power of reducing sugar by the action of alkali, effecting a rearrangement of the glucose and fructose molecules and their transformation into an inactive mixture of glucose, fructose and mannose. The various analytical methods are reviewed in Browne's Handbook of Sugar Analysis, p. 302, also in Louisiana Bulletin No. 135, p. 47, where a modification of the method of Pellet and Lameland, employing hydrogen peroxide in addition to alkali is described by Cross and Taggart. They found Pellet's method unreliable, but although their own modification gave results in close agreement with Clerget's inversion method for sugar house products, it yielded too low figures with pure sucrose. This fact, illustrated in Tables I and II, might be

TABLE I

25 cc of a sucrose solution mixed with varying amounts of reducing sugar (glucose-fructose) were heated with 2 cc NaOH (40° Be) and 45 cc H_2O_2 (3%) at 55°C for 20 minutes, on cooling neutralized and made up to 100 cc.

g Reducing Sugar	Polarization V
0.	24.25
0.25	24.45
0.5	24.85
1.0	25.05
1.5	25.05
2.0	25.05
0. Without NaOH, H_2O_2	24.7

TABLE II

Solution A containing 5% fructose
" B " 12.5g invert sugar syrup (*) 77% (with 3% sucrose) in 200 cc
" C " 25% sucrose
were mixed in varying proportions and heated with 2 cc NaOH (40° Be) and 45 cc H_2O_2 (3%) in boiling water bath for 20 minutes, on cooling neutralized and made up to 100 cc.

Mixture	Polarization °V
25 cc A	0.
50 cc A	0.
25 cc B	0.2
50 cc B	0.38
25 cc C	22.1
25 cc A + 25 cc C	23.4
25 cc B + 25 cc C	23.4
25 cc C without H_2O_2	24.6
25 cc C without NaOH, H_2O_2	24.8

* An invert sugar syrup prepared by inversion of 1000g sucrose +250g water with ½ cc HCl at 95-97° (Z. Zuckerind. Böhmen, 1915, p. 282); it contained 2.9 per cent sucrose according to Clerget's method.

explained by a slight destruction of sucrose by hydrogen peroxide in alkaline solution analagous to the action of Fehling solution on sucrose in the presence of reducing sugars. It was therefore sought to avoid the use of an oxidant for the decoloration of the dark reaction products formed on heating reducing sugars with alkali, and sulphur dioxide and sodium hydrosulfite were substituted for this purpose. The detail of the method employed is as follows:

The normal weight of material (= 26g.) is dissolved in a 200 cc flask, the solution clarified with lead subacetate, filled up to the mark and filtered. The excess of lead is removed from the filtrate by gaseous SO_2 or dry K_2HPO_4.

A 50 cc measuring flask is filled up to the mark with the filtrate and 1 cc of KOH (36°Be.) added. After heating in a boiling water bath for about 45 minutes, a few crystals of Blankit are added to the hot solution, which after cooling is neutralized with SO_2 gas. As soon as the liquid becomes acid, the color brightens up very considerably. There is no danger from inversion by this way of procedure, as is shown in Tables XI and XIV. The flask is filled up to the 55 cc mark and the solution polarized. The following table gives a comparison of results by Clerget-Herzfeld's inversion method and the one just described:

TABLE III

Product	% Sucrose by Clerget	% Sucrose by above method
Blackstrap	33.1	31.24
Blackstrap	33.0	32.12
Blackstrap	34.0	32.46
Blackstrap	33.8	32.12
1st. Molasses	46.5	45.22
1st. Molasses	47.5	46.64
1st. Molasses	47.3	46.74
Raw Sugar	91.9	92.8
Raw Sugar	96.85	96.8
RawS ugar	91.4	91.1

The results agree fairly closely for the raw sugars, but regular differences of 1 to 2 per cent occur with molasses. These must be ascribed to a residual levorotation of reducing sugar. This explanation becomes still more apparent from the following analysis of artificial mixtures of sucrose and reducing sugars:

TABLE IV

Solution A containing 200g sucrose (= 76.78% of normal weight) in 500 cc

" B " 62.5g invert sugar syrup of 77% (+2.9% sucrose) in 500 cc

were mixed in varying proportions and the volumes completed to 200 cc:

Mixture	% Invert Sugar	% SUCROSE		
		Calculated	By inversion	By above method
50 cc A		76.78	76.7	
100 cc B			1.4	*
50 cc A+100 cc B	5	78.18	78.06	76.12
50 cc A+80 cc B	4	77.9	77.9	76.12
50 cc A+60 cc B	3	77.62		76.12
50 cc A+40 cc B	2	77.34	77.2	76.12
50 cc A+20 cc B	1	77.06	77.3	76.34

* Levorotary (compensating 1.32% sucrose.)

The results obtained by the direct method show differences from the actual sucrose contents varying from 0.7 per cent for a concentration of 1 per cent invert sugar to 2 per cent for a concentration of 5 per cent invert sugar. We now proceeded to ascertain by what change of the working conditions a more complete reduction of the invert sugar rotation could be accomplished. 50 cc of a solution of the above mentioned invert sugar were heated for 45 minutes with varying amounts of alkali as follows, a trace of hydrosulfite added, after cooling neutralized with SO_2 and made up to 50 cc.

TABLE V

gKOH	Polarization °V	
0.	- 4.95 (32°C)	
0.045	- 2.4	
0.09	- 2.4	
0.135	- 2.4	
0.18	- 2-1	- 1.75
0.22		
0.27	- 1.65	
0.36	- 1.5	
0.45	- 1.4	
0.54	- 1.0	
0.63	- 1.0	
0.67		- 1.00
0.9		- 0.6
1.12		- 0.6
1.35		- 0.7
1.8		- 0.7
2.25		- 0.6

The above results show that the reading of a 5 per cent invert sugar solution cannot be reduced to 0° ; the minimum levorotation is reached with about 2 per cent KOH (36° Be.) to 50 cc of sugar solution, stronger concentrations of alkali producing no apparent effect on the small residuary rotation.

With weaker invert sugar solutions the results were as follows:

TABLE VI

50 cc of invert sugar solution of various concentrations were heated as above with 1 cc of NaOH (40°Be) = 0.48g NaOH, decolorized, neutralized and made up to 55 cc.

% Invert Sugar	Polarization °V
5	- 1.6
4	- 1.2
3	- 0.8
2	- 0.6
1	- 0.3
0.5	- 0.3
0.25	- 0.1

The results in Table III obtained with raw sugars correspond to the weaker concentrations of invert sugar in Table VI; the error thus produced is not large enough to materially affect the results. The higher concentrations of invert sugar in Table VI correspond to the results obtained for molasses; the residuary rotation is large enough to introduce a serious error. A greater dilution of molasses to a reducing sugar content of about 1 per cent would involve a corresponding multiplication of a smaller error and lead ultimately to exactly the same result. To make effective use of the method it would be necessary to apply corrections to its results varying with the concentrations of reducing sugar. They would have to be fixed empirically for the concentrations of alkali employed at a certain temperature. Prolonged heating does not alter the results; the following table shows that the minimum of levorotation is reached after 15-20 minutes:

TABLE VII

50 cc of an invert sugar solution (about 5%) were heated with 0.45g KOH and on neutralization made up to 55 cc.

Time of Heating	Polarization °V
Not heated	- 4.9 (at 30°C)
15 min.	- 1.1
30 min.	- 1.0
45 min.	- 1.1
75 min.	- 1.0
105 min.	- 1.0
135 min.	- I.0

The destruction of the rotary power of glucose also results in a slight levorotation; a minimum is apparently reached more easily than for fructose:

TABLE VIII

50 cc of 5% glucose, fructose and invert sugar solutions were heated with varying amount of alkali as follows:

gNaOH added	Polarization °V		
	Glucose	Fructose	Invert Sugar
0.	+0.5	- 22.7	- 5.0
0.25	- 0.4	- 10.5	
0.12	- 0.4	- 3.1	
0.25	- 0.3	- 1.1	- 0.7
0.37	- 0.2	- 0.9	
0.50	- 0.2	- 0.7	- 0.7
0.75		- 0.6	- 0.5
1.00		- 0.6	- 0.4
1.25		- 0.6	. 0.4

An effect of the salts resulting from the neutralization of alkali on the saccharimeter reading of sucrose is hardly noticeable with quantities of alkali used in the method described above; but it becomes very apparent at higher concentrations:

TABLE IX

The following quantities of KOH were added to 50 cc of sucrose solution and the volumes completed to 55 cc.

gKOH	Polarization °V		
	Alkaline	Neutralized by SO_2	Neutralized by SO_2
0.		90.45	45.6
0.22			45.3
0.45			45.3
*0.67	87.30	90.05	
0.90			45.95
1.12	85.65	89.9	
1.35	84.35	89.3	
†1.8	82.75	89.5	

* Pellet's concentration.
† Cross' concentration.

Besides this effect due to the presence of neutral alkali salts a further decrease of the sucrose rotation is evidently due to the action of hot alkali (*). Thus heated (for 45 minutes) pure sucrose solutions acquire a yellow color, increasing with the amount of alkali. This coloration cannot result from the destruction of traces of invert sugar, as the polarization is noticably lower on neutralization, than that of a corresponding solution not heated.

TABLE X

The following amounts of alkali were added to 50 cc of sucrose solution and the volumes completed to 55 cc.

gNaOH added	Polarization °V			
	Not Heated		Heated	
	Alkaline	Neutralized (SO_2)	Alkaline	Neutralized (SO_2)
0.		45.41		
0.5	43.05	45.0	43.0	44.85
1.0	41.5	44 6		44.25
1.5	40.5	44.5	39.5	43.85
2.0	39.9	44 1	38.5	43.3

The explanation might be offered, that this difference is due to incomplete neutralization and the effect of alkali bicarbonate, which would result from a CO_2 content of the alkali with phenolphthalein as an indicator. The results were, however, not materially affected by a very distinct acidification. This acidity was not strong enough to cause inversion, the polarimeter reading remaining quite constant.

TABLE XI

The following quantitles of alkali were added to 50 cc of sucrose solution and on neutralization with acetic acid the volumes completed to 100 cc.

added gNaOH	Polarization °V				
	Not Heated Neutralized	Heated Neutralized	Not Heated Neutralized	Heated Neutralized	Acidified
0.	86.95	86.6			
0.33	86.85	86.0	61.2	61.0	61.1
0.67	86.6	85.3	61.1	60.85	60.8
1.0	85.85	84.55	60.9	60.6	60.5
1.33	85.75		60.75	60.25	60.05
1.67			60.8	60.15	60.0

Low results obtained by Cross' modification for pure sucrose could partially be explained by the influence of neu-

(*) Compare Herzfeld, Z. V. D. Zuckerindustrie, 43,735.

tral alkali salts as the concentration of NaOH in his method is 2.5% NaOH; while Pellet's was only 1%.

A second correction would necessarily have to be applied in the application of these methods varying according to the concentration of alkali and sugar solution; this may be computed from the following tables:

TABLE XII

Varying amounts of alkali neutralized with SO_2 or CH_3COOH were added to 25 cc of sucrose solution and the volumes completed to 50 cc.

added gNaOH	Neutral by SO Polarization °V	Decrease of rotation 100°V=	Neutral by CH_3COOH Polarization °V	Decrease of rotation 100 °V=
0.	82.75			
0.3	82.25	99.43	82.60	99.82
0.6	81.90	98.97	82.35	99.52
0.9	81.65	98.67	82.10	99.23
1.2	81.20	98.13	81.95	99.03
1.5	80.75	97.58	81.90	98.97

TABLE XIII

25 cc of sucrose solutions of varying concentration are made up to 50 cc with and without 1.25g NaOH, neutralized by SO_2.

Polarization °V		Decrease of rotation 100°V =
Without Alkalisulfite	With	
101.65	99.8	98.2
61.8	60.75	98.3
40.8	40.1	98.3
19.6	19.2	98.0
10.2	10.0	98.0

Sodium acetate, resulting in Pellet's and Cross and Taggart's method from the neutralization of the alkali, has a weaker but still very noticeable effect on the sucrose rotation than sodium sulphite. Incomplete neutralization, leaving the solution alkaline would of course result in a very serious error, as shown in Table X. On the other hand, inversion on very strong acidification is not rapid enough to affect the results if the solution is not allowed to become warm and is polarized in a few minutes. On the contrary, a distinct increase in the saccharimeter reading is first noticed as Na_2SO_3 is being changed to $NaHSO_3$:

TABLE XIV

Equal volumes (25 cc) of a sucrose solution and an alkali sulfite solution of varying acidity (prepared by acidifying a solution of 12.5g NaOH in 250 cc with gaseous SO_2) were mixed and made up to the same volume.

Acidity of mixed sucrose solution 1 cc = cc $\frac{n}{100}$	Polarization °V
0.	80.6
0.8	80.6
2.8	80.6
15.9	80.8
19.7	80.9
31.0	81.0
36.0	81.0
39.5	80.95
0. (without alkali)	81.4
0.3	75.5
3.4	76.05
8.1	76.05
14.0	76.4
0. (without alkali)	76.8

The summary of the above experiments is that three sources of error are inherent in the direct methods of sucrose determination in the presence of reducing sugar:

(1) The residuary levorotation. This introduces a considerable error, especially with material high in reducing sugar and with methods using a weak concentration of alkali.

(2) Stronger concentration of alkali reduces this levorotation but introduces a second serious error, due to the decrease of sucrose rotation by the resulting alkali salts.

(3) Oxidation of sucrose by heating in alkaline solution, especially in the hydrogen peroxide methods.

Apparently correct results are occasionally obtainable due to a compensation of these errors.